

ANTIMETASTATIC ACTIVITY OF *HOLOTHURIA POLII* TRITERPENE GLYCOSIDES FROM TURKISH COASTLINE

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Abstract

Sea cucumbers have special secondary metabolites called triterpene glycosides. These metabolites show some important bioactivities. In the present work, triterpene glycosides from *Holothuria polii* were isolated by using HPLC and characterized by MALDI-TOF/MS. Antimetastatic activity of partially isolated triterpene glycosides was carried out. Triterpene glycosides could be alternative natural anti-metastatic agents to current synthetic agents.

Keywords: *Biotechnologies, Aegean Sea, Echinodermata*

Introduction

Holothuria polii Delle Chiaje, 1823 is a common, white spot sea cucumber which is widely distributed in the Mediterranean sea from 0 to 250 m deep [1]. Holothurians secrete so called glycoside based triterpenes as secondary metabolites. The structures of these molecules are changeable from species to species and from region to region. Antifungal, antiproliferative, anti-inflammatory, anti-thrombotic, antibacterial, apoptotic, cytotoxic, hemolytic, cytostatic and immunomodulatory properties of triterpene glycosides have so far been reported in scientific papers [2]. Metastasis, which is known as permeation of cancer cells away from the origin, is the most effective factor on both cancer progression and deaths. Tumor metastasis which includes cell proliferation, proteolytic digestion of extracellular matrix, cell migration to circulatory system and tumor growth in metastatic regions, is a multistep and complex process [3]. Novel treatment methods by using natural compounds are of great importance for metastasis process. In this study, it was aimed to investigate the antimetastatic activity of *H. polii* triterpene glycosides on T84 cell line.

Materials and Method

H. polii samples were collected from Dikili (Aegean Sea, Turkey) at a depth of 0-2 m. After the samples were transferred to laboratory within the fresh-sea water, they were frozen at -18 °C to kill [4]. In order to isolate the triterpene glycosides, the body walls of the *H. polii* were homogenized and extracted and semi-purified by using the method of Bondoc et al. 2013. Finally, the isobutanol phase was evaporated and pH 7.4 HPLC grade water was added. The chromatographic separations were performed with Shimadzu LC-20 HPLC system connected to SPD-20A UV detector. Fractionation of triterpene glycosides was performed on Inertsil C18 HPLC column (GL Sciences), (250 mm x 4.6 mm, 5 µm). The analytes were eluted by using non-linear gradient of methanol (A) and water (B) (0 min, 10% A; 6 min, 60% A; 13 min, 60% A; 15 min, 10% A) at a flow rate of 1 mL/min. Column temperature was 27°C [5]. Also, the fractions were analysed by using MALDI-MS/MS on positive ion mode. In order to determine the total saponin concentrations in each fraction, vanillin-sulfuric acid colorimetric method [6] was carried out. xCELLigence Real-Time Cell Analysis (RTCA) was used in cell migration-invasion assays on T84 cell line. Data analysis was carried out with RTCA software vs. 1.2.1.

Results

HPLC chromatogram of semi-purified triterpene glycosides was given in Figure 1. The retention times of fraction A, B, C and D were 9.6, 10.6, 11.7 and 13.4 min, respectively. In fractions, total saponin were found as 296.797 mg/L, 391.892 mg/L, 236.737 mg/L and 341.842 mg/L for fraction A, B, C and D, respectively. Although in fraction C contains both holothurin A (1243.50 m/z) and a novel isomer (1127.50 m/z), fraction D contains only the novel isomer. According to the antiproliferation results of fraction A-D on T84, IC₅₀ values were found as 38.92±6.03, 37.99±3.81, 21.46±1.49 and 17.64±1.10 mg/L, for fraction A, B, C and D, respectively. The most effective fraction on antiproliferation for T84 cell line was found as fraction D. Also, the invasion and migration result reveals that 35 and 10 ppm of *H. polii* extract show antimetastatic activity on T84 cell line.

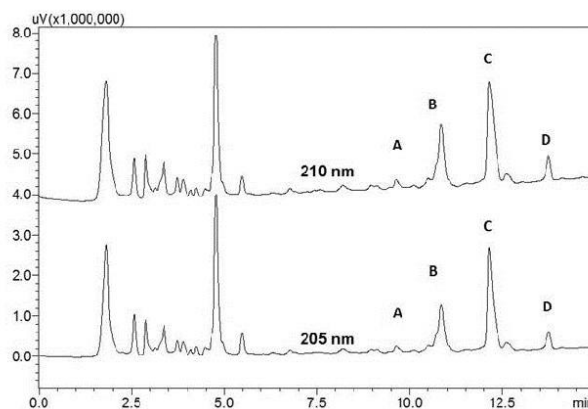


Fig. 1. A sample HPLC chromatogram of semi-purified triterpene glycosides at 205 and 210 nm.

Conclusion

Triterpene glycosides are important bioactive metabolites which are drug candidates against metastasis. Thus, *H. polii* could be evaluated as a novel, promising antimetastatic agent source.

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